7 CHAPTER 7 Antigens: Triggers of Acquired Immunity

7.1 KEY POINTS

- The acquired immune system is optimized to recognize microbial macromolecules.
- The best antigens are large, complex, stable, foreign proteins.
- Small molecules with a mass of less than 5000 kDa are usually poor antigens.
- Small molecules may be made antigenic by conjugating them to large proteins. Small molecules used as antigens in this way are called haptens.
- The immune system recognizes distinct areas on the surface of foreign antigens. These are called antigenic determinants or epitopes.

Until now we have considered only the body's innate reactions to microbial invasion. Innate responses are triggered by recognition of pathogen-associated molecular patterns (PAMPs) such as CpG DNA and lipopolysaccharide. The triggering of inflammation and the mobilization of phagocytic cells such as neutrophils and macrophages induced by these molecules contribute to the rapid destruction of microbial invaders. Although this may be sufficient to protect the body, it cannot always provide the level of immunity required to ensure resistance to infection. Thus a more potent immune response should recognize all the foreign molecules on an invading microbe. In addition, an ideal defensive response would be able to learn from this experience and, given time, develop more efficient procedures to combat subsequent invasions. This adaptive response is the function of the acquired immune system.

During an acquired immune response, molecules from invading organisms are captured, processed, and presented to the cells of the immune system. When appropriately presented, these antigens will trigger a powerful protective immune response that ensures an animal's survival. In addition, the immune system “remembers” these antigens and responds even more effectively if it encounters them subsequently.

7.2 ANTIGENS

Since the function of the acquired immune system is to defend the body against invading microorganisms, it is essential that these organisms be recognized as soon as they invade the body. The body must be able to recognize that these are foreign (and dangerous) if they are to stimulate an immune response. The innate immune system recognizes a limited number of PAMPs that are characteristic of major groups of pathogens. The acquired immune system, however, can recognize and respond to a vast array of foreign molecular structures. These molecular structures are called antigens.

7.3 MICROBIAL ANTIGENS

7.3.1 Bacterial Antigens

Bacteria are ovoid or spherical organisms consisting of a cytoplasm containing the essential elements of cell structure surrounded by a lipid-rich cytoplasmic membrane (Figure 7-1). Outside the cytoplasmic membrane is a thick, carbohydrate-rich cell wall. The major components of the bacterial surface thus include the cell wall and its

associated protein structures—the capsule, the pili, and the flagella. The cell wall of Gram-positive organisms is largely composed of peptidoglycan (chains of alternating *N*-acetyl glucosamine and *N*-acetyl muramic acid cross-linked by short peptide side chains; see Chapter 2, Figure 2-2). Gram-positive cell walls also contain lipoteichoic acids that are involved in transport of ions across the cell wall. The cell wall in Gram-negative organisms consists of a thin layer of peptidoglycan covered by an outer membrane consisting of a lipopolysaccharide. Most of the antigenicity of Gram-negative bacteria is associated with this component. It consists of an oligosaccharide attached to a lipid (lipid A) and to a series of repeating trisaccharides. The structure of these trisaccharides determines the antigenicity of the organism. Many bacteria are classified according to this antigenic structure. For example, the genus *Salmonella* contains a major species, *Salmonella enteritica,* that is then divided into more than 2300 serovars based on antigenicity. These polysaccharide antigens are called O antigens. When an animal is infected, the outer cell wall lipopolysaccharides of Gram-negative bacteria bind to toll-like and other pattern-recognition receptors and so induce the production of a mixture of cytokines. Because these cytokines are toxic, bacterial lipopolysaccharides are also called endotoxins.

Bacterial capsules consist of polysaccharides (except in *Bacillus anthracis,* where the capsule consists of proteins). These polysaccharides are usually good antigens. Capsules protect bacteria against phagocytosis (see Chapter 3), and anticapsular antibodies can protect an infected animal. Capsular antigens are collectively called K antigens.

Pili and fimbriae are short projections that cover the surfaces of some Gram-negative bacteria; they are classified as F or K antigens. Pili attach the bacteria to other bacteria and play a role in bacterial conjugation. Fimbriae attach bacteria to surfaces. Antibodies to fimbriae may have an important protective function since they can prevent bacteria from sticking to body surfaces. Bacterial flagella consist of a single protein called flagellin. Flagellar antigens are collectively called H antigens.

Other significant bacterial antigens include the porins, the heat-shock proteins, and the exotoxins. The porins are proteins that form the pores on the surface of Gram-negative organisms. Heat-shock proteins are generated in large numbers in stressed bacteria. The exotoxins are toxic proteins secreted by bacteria or released into the surrounding environment when they die. Exotoxins are highly immunogenic proteins that, when an animal is infected, stimulate the production of antibodies called antitoxins. Many exotoxins, when treated with a mild protein-denaturing agent such as formaldehyde, lose their toxicity but retain their antigenicity. Toxins modified in this way are called toxoids. Toxoids may be used to prevent disease caused by toxigenic bacteria such as *Clostridium tetani*. Bacterial nucleic acids rich in unmethylated CpG sequences serve both as effective antigens for the acquired immune system and as potent stimulators of innate immunity acting through toll-like receptors.

7.3.2 Viral Antigens

Viruses are very small organisms that can grow only inside living cells. They are thus “obligate,” intracellular parasites. Viruses have a relatively simple structure consisting of a nucleic acid core surrounded by a protein layer (Figure 7-2). This protein layer is termed the capsid, and it consists of multiple subunits called capsomeres. The capsid proteins are good antigens, highly capable of stimulating antibody formation. Some viruses may also be surrounded by an envelope containing lipoproteins and glycoproteins. A complete viral particle is called a virion. When a virus infects an animal, the proteins in the virions act as antigens

FIGURE 7-1 The structure of a typical bacterium and the location of its most important antigens.

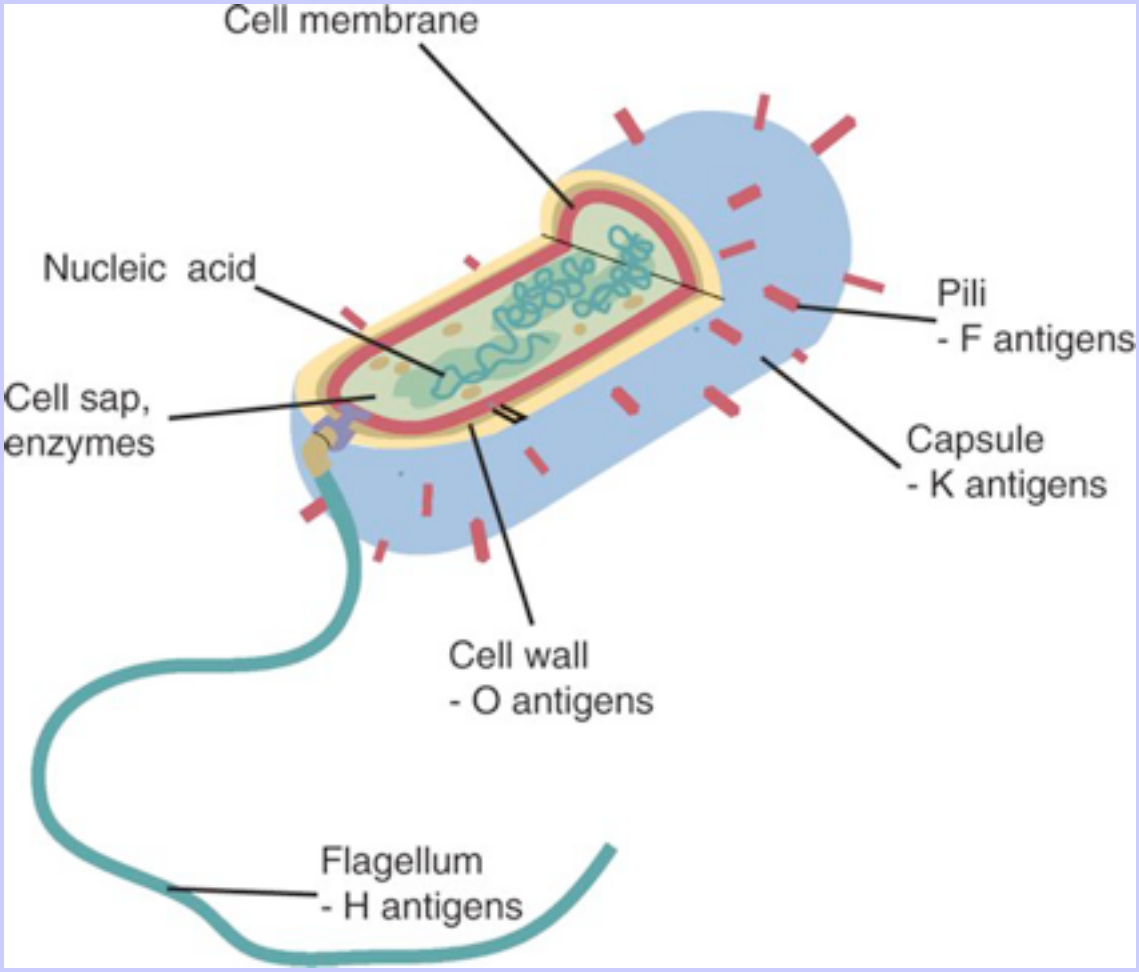

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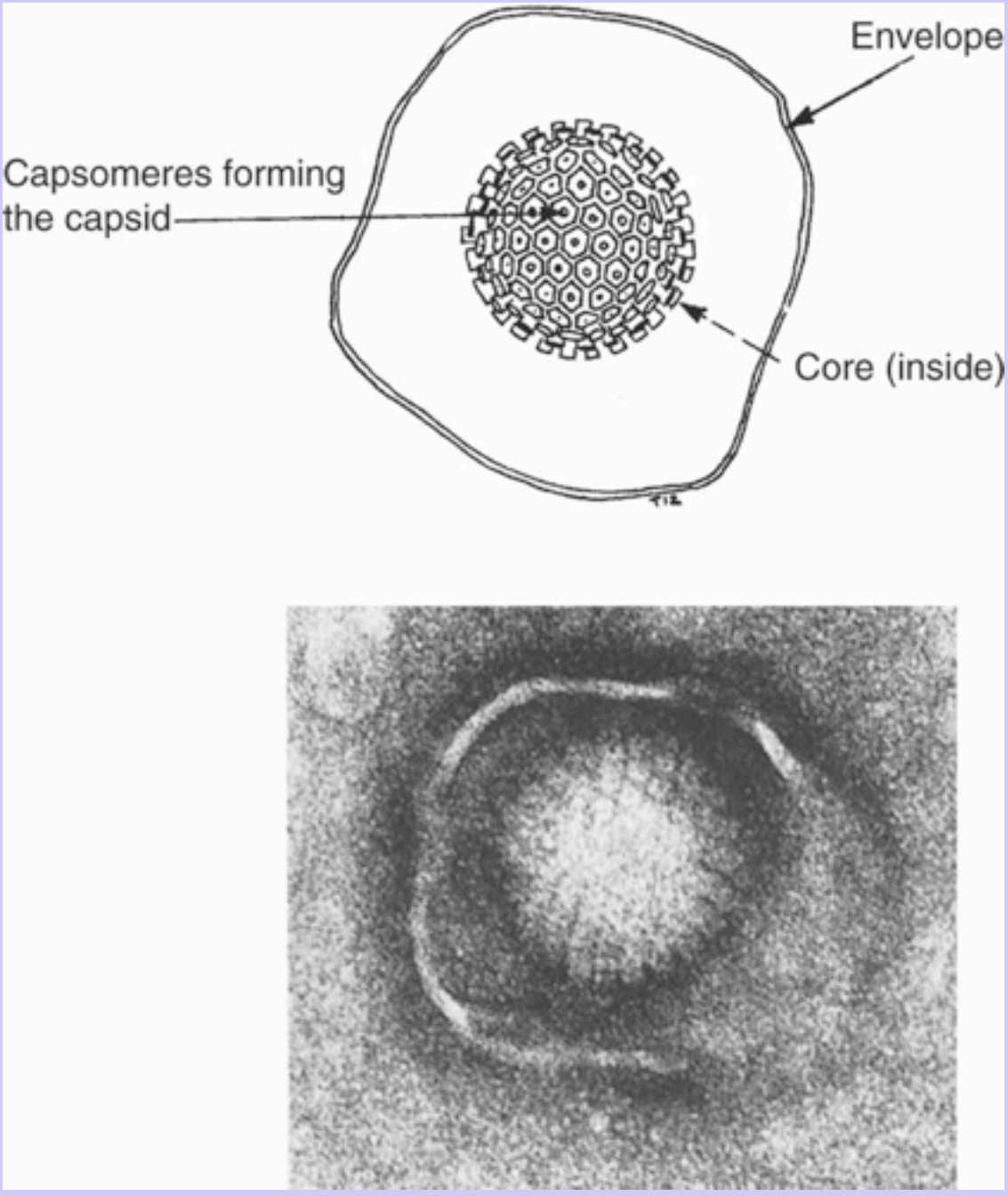
FIGURE 7-2 The structure of a virus. This is an electron micrograph of equine herpesvirus type 4 magnified 184,000 times. The virus is negatively stained—that is, the electron-dense "dye" has filled the low areas on the virion, leaving the higher areas unstained. (Courtesy Dr. J. Thorsen.)



and trigger an acquired immune response. Viruses, however, are not always found free in the circulation but live within cells, where they are protected from the unwelcome attentions of antibodies. Indeed, viral nucleic acid can be integrated into a cell's genome. In this situation, the viral genes code for new proteins, some of which are carried to the surface of infected cells. These proteins, although they are synthesized within an animal's cells, are still considered foreign and can provoke acquired immunity. These newly synthesized foreign proteins are called endogenous antigens to distinguish them from the foreign antigens that enter from the outside and are called exogenous antigens.

7.3.3 Other Microbial Antigens

In addition to bacteria and viruses, animals may be invaded by fungi, protozoan parasites, and even by parasitic worms (helminths). Each of these organisms consists of many different structures composed of proteins, carbohydrates, lipids, and nucleic acids. Some of these can serve as antigens and trigger acquired immunity. However, their antigenicity does vary, and the immune responses triggered by these organisms do not always protect an animal or eliminate the invader.

7.4 NONMICROBIAL ANTIGENS

Invading microorganisms are not the only source of foreign material entering the body. Food may contain foreign molecules that under some circumstances trigger immune responses and cause an allergic reaction. Likewise, inhaled dusts can contain antigenic particles such as fungal spores or pollen grains, and antigens from these may enter the body through the respiratory system. Foreign molecules may be injected directly into the body through a snake or mosquito bite, or they may be injected deliberately by a veterinarian administering a vaccine or a blood transfusion. Furthermore, foreign proteins may be injected into animals for experimental purposes. Organ grafts are an effective way of administering a large amount of foreign material to an animal.

7.4.1 Cell Surface Antigens

The cytoplasmic membrane of every mammalian cell consists of a mosaic of protein molecules immersed in a fluid lipid bilayer. Most of these proteins can act as antigens if they are injected into an animal of another species or even into a different animal of the same species. For example, glycoproteins known as blood group antigens are found on the surface of red blood cells. Early attempts to transfuse blood between unrelated individuals usually met with disaster because the transfused cells were rapidly destroyed even though the recipient had never before received a transfusion. Investigation revealed that the problem was due to the presence of naturally occurring antibodies against these red cell glycoproteins (see Chapter 26).

Nucleated cells, such as leukocytes, possess hundreds of different protein molecules on their cytoplasmic membrane. These proteins are good antigens and readily provoke an immune response when injected experimentally into an animal of a different species. These surface molecules are classified by the cluster of differentiation system (see Chapter 2). Other cell surface proteins may provoke an immune response (such as graft rejection) if transferred into a genetically different individual of the same species. The most important cell surface proteins that trigger graft rejection are called major histocompatibility complex molecules. These molecules are of such importance in immunology that they warrant a complete chapter of their own (see Chapter 9).

7.4.2 Autoantigens

In some situations (and not always abnormal ones), an immune response may be directed against normal body components. This is called an autoimmune response. Antigens that induce this autoimmunity are called autoantigens. They can include hormones such as thyroglobulin; structural components such as basement membranes; complex lipids such as myelin; intracellular components, such as the mitochondrial proteins, nucleic acids, or nucleoproteins; and cell surface proteins, such as hormone receptors. The production of these autoantibodies and the consequences of this production are discussed in detail in Chapter 31.

7.5 WHAT MAKES A GOOD ANTIGEN?

Molecules vary in their ability to act as antigens (their antigenicity) and stimulate an immune response (Figure 7-3). In general, foreign proteins make the best antigens, especially if they are big. (A molecular weight greater than 1 kDa is best.) Many of the major antigens of microorganisms such as the clostridial toxins, bacterial flagella, virus capsids, and protozoan cell membranes are proteins. Other important antigenic proteins include components of snake venoms, serum proteins, cell surface proteins, milk and food proteins, hormones, and even antibody molecules themselves.

Simple polysaccharides, such as starch or glycogen, are not good antigens simply because they are degraded before the immune system has had time to respond to them. More complex carbohydrates, however, may be of immunological importance, especially if bound to proteins. These include the major cell wall antigens of Gram-negative bacteria and the blood group antigens of red blood cells. Many of the so-called natural antibodies found in the serum of unimmunized animals are directed against polysaccharides and probably arise as a result of exposure to glycoproteins or carbohydrates from the normal intestinal flora or from food. To this extent they can also be considered to be part of the innate immune system.

Lipids tend to be poor antigens because of their wide distribution, relative simplicity, structural instability, and rapid metabolism. Nevertheless, when linked to proteins or polysaccharides, lipids may be able to trigger immune responses.

Mammalian nucleic acids are very poor antigens because of their relative simplicity and flexibility and because they are very rapidly degraded. Microbial nucleic acids, on the other hand, have a structure very different from that found in eukaryotes with many unmethylated CpG sequences. As a result, they can stimulate a potent acquired immune response. It is perhaps for this reason that autoantibodies to nucleic acids are a characteristic feature of several important autoimmune diseases (see Chapter 33).

Proteins are the most effective antigens because they have properties that best trigger an immune response. (More correctly, the acquired immune system is optimized to trap, process, and then recognize foreign proteins.) Thus large molecules are better antigens than small molecules, and proteins can be very large indeed (Figure 7-4). For example, hemocya

FIGURE 7-3 The factors that significantly influence the antigenicity of a molecule. Of these, either excessive or insufficient stability will reduce antigenicity. The best antigens are large, complex, and foreign. However, their ability to stimulate an immune response is also determined by their route of administration, by the amount of antigen administered, and by the genetic makeup of the immunized animal.

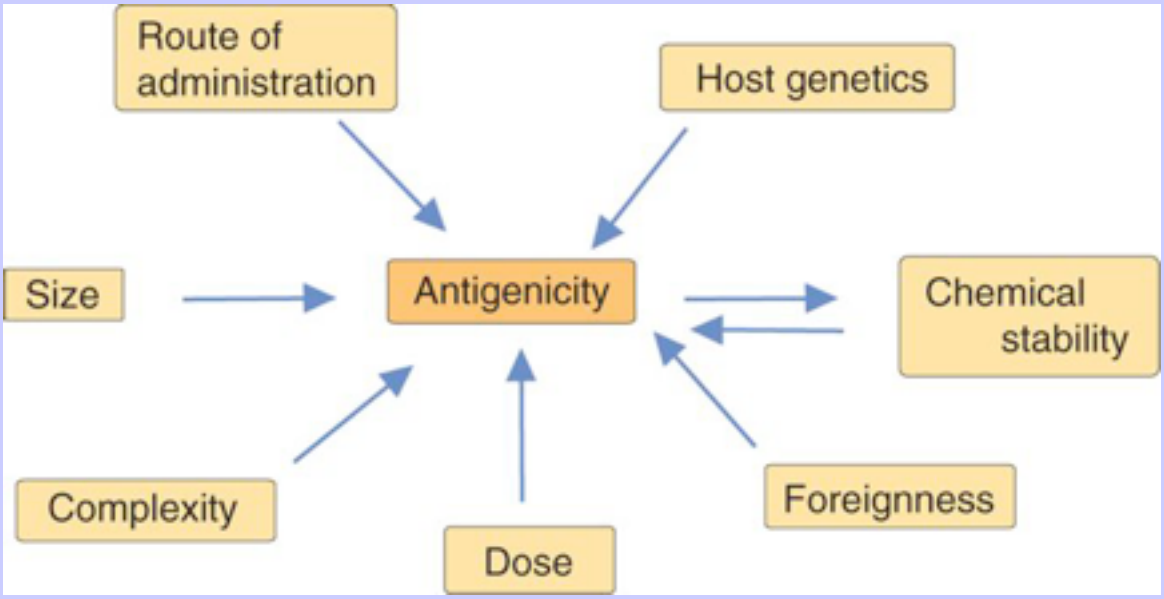
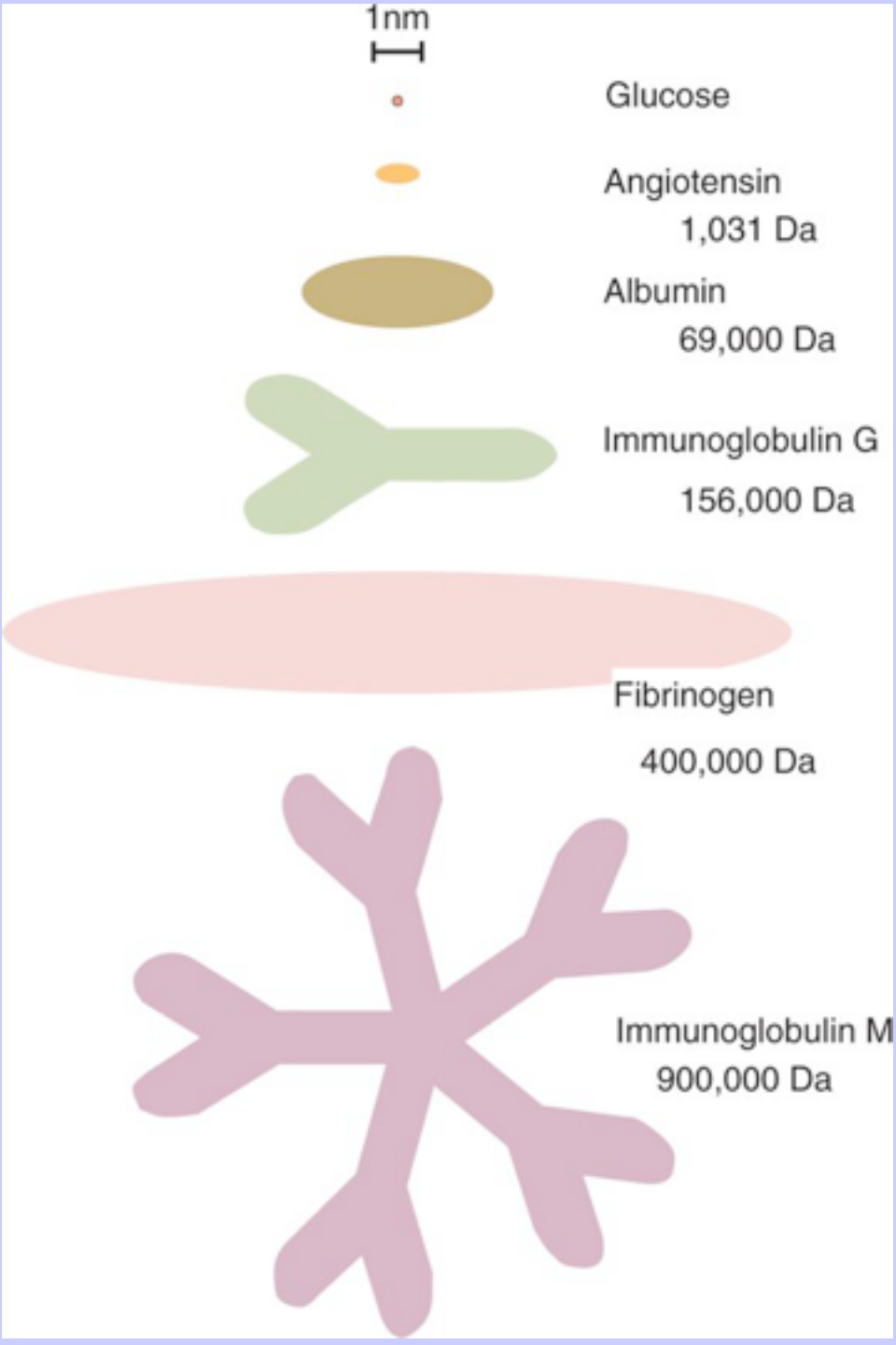


FIGURE 7-4 The relative sizes of several significant antigens. Size does matter—big molecules are generally much more antigenic than small molecules. Molecules as small as angiotensin are poor antigens.



nin, a very large protein from invertebrate blood (670 kDa) is a potent antigen. Serum albumin from other mammals (69 kDa) is a fairly good antigen but may also provoke tolerance. The small peptide hormone angiotensin (1031 Da) is a poor antigen. Similarly, the more complex an antigen is, the better. For example, starch and other simple repeating polymers are poor antigens, but complex bacterial lipopolysaccharides are good. Complex proteins containing many different amino acids, especially aromatic ones, are better antigens than large, repeating polymers, such as the lipids, carbohydrates, and nucleic acids.

Structural stability is an important feature of good antigens, especially those that trigger antibody responses. To bind to a foreign molecule, the cell surface receptors of the immune system must recognize its shape. Consequently, highly flexible molecules that have no fixed shape are poor antigens. For example, gelatin, a protein well known for its structural instability (which is why it can wobble), is a poor antigen unless it is stabilized by the incorporation of tyrosine or tryptophan molecules, which cross-link the peptide chains. Similarly, flagellin, the major protein of bacterial flagella, is a structurally unstable, weak antigen. Its stability, and thus its antigenicity, is greatly enhanced by polymerization. Remember too that the route of antigen administration, its dose, and the genetics of the recipient animal also influence antigenicity.

Clearly not all foreign molecules can stimulate an immune response. Stainless steel bone pins and plastic heart valves, for example, are commonly implanted in humans without triggering an immune response. The lack of antigenicity in the large organic polymers, such as the plastics, is due not only to their molecular uniformity but also to their inertness. These polymers cannot be degraded and processed by cells to a form suitable for triggering an immune response. Conversely, since immune responses are antigen-driven, foreign molecules that are unstable and destroyed very rapidly may not persist for a sufficient time to stimulate an immune response.

7.5.1 Foreignness

The cells that respond to antigens (antigen-sensitive cells) are selected so that their receptors do not normally bind to molecules originating within an animal (self-antigens). They will bind and respond, however, to foreign molecules that differ even in minor respects from those normally found within the body. This lack of reactivity of the acquired immune system to normal body components occurs because cells whose receptors bind self-antigens are selectively killed. During their development, the cells are exposed to self-antigens, and those cells that respond are killed in a process called negative selection.

The immunogenicity of a molecule also depends on its degree of foreignness. The greater the difference in molecular structure between a foreign antigen and an animal's own antigens, the greater will be the intensity of the immune response. For example, a kidney graft from an identical twin will be readily accepted because its proteins are identical to those on the recipient's own kidney. A kidney graft from an unrelated animal of the same species will be rejected in about 10 days unless drugs are used to control the rejection. A kidney graft between different species such as one from a pig to a dog will be rejected within a few hours despite the use of immunosuppressive drugs.

7.6 EPITOPES

Foreign particles, such as bacteria, nucleated cells, and red blood cells, are a complex mixture of proteins, glycoproteins, polysaccharides, lipopolysaccharides, lipids, and nucleoproteins. The acquired immune response against such a foreign particle is therefore a mixture of many simultaneous immune responses against each of the foreign molecules in the mixture.

A single large molecule such as a protein can also be shown to stimulate multiple immune responses. Large molecules have specific regions, against which immune responses are directed. These regions, usually on the surface of the molecule, are called epitopes, or antigenic determinants (Figure 7-5). In a large, complex protein molecule, many different epitopes may be recognized by the immune system, but some are much more immunogenic than others. Thus animals may respond to a few favored epitopes, and the remainder of the molecule may be virtually nonimmunogenic. Such epitopes are said to be immunodominant. In general, the number of epitopes on a molecule is directly related to its size: there is usually about 1 epitope for each 5 kDa of a protein. When we describe a molecule as "foreign," therefore, we are implying that it contains epitopes that are not found on self-antigens. The cells of the immune system recognize and respond to foreign epitopes. A good example of a well-defined epitope is the peptide "proline-glutamic acid-proline-lysine" that binds to antibodies against the bacterium *Streptococcus equi*. Presumably the shape of this peptide is identical to the major antigenic determinant on *S. equi*.

7.6.1 Haptens

A small molecule such as a drug or a hormone with a molecular weight of less than 1 kDa is far too small to be appropriately processed and presented to the immune system. As a result, it is not immunogenic. If, however, the small molecule is chemically linked to a large protein molecule, completely new epitopes will be formed on the surface of the larger molecule (Figure 7-6). If this molecular complex is injected into an animal, immune responses will be triggered against all its epitopes. Some of the antibodies made in response to the complex will be directed against new epitopes formed by the small molecule. Small molecules or chemical groups that can function as epitopes only when bound to other larger molecules are called haptens (in Greek, *haptein* means "to grasp or fasten"). The antigenic molecule to which the haptens are attached is called the carrier. Many drug allergies occur because the drug molecules, although small, can bind covalently to normal body proteins and so act as haptens.

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By using haptens of known chemical structure, it is possible to study the interaction between antibodies and epitopes in great detail. For example, antibodies raised against one hapten can be tested for their ability to bind to other, structurally related molecules. Simple tests have shown that any alteration in the shape, size, or charge of a hapten alters its ability to bind to antibodies. Even very minor modifications to the shape of a hapten, such as the difference between stereoisomers, may influence its ability to be bound by an antibody. Since an enormous variety of potential haptens exists, and since each hapten can provoke its own specific antibodies, it follows that animals must be able to generate an extremely large variety of specific antibody molecules. This enormous diversity enables animals to successfully fight the multitude of pathogenic microbes.

7.6.2 Some Examples of Haptens

Although the concept of haptens and carrier molecules provides the basis for much of our knowledge concerning the specificity of the antibody response, haptens may also be of clinical importance. For example, the antibiotic penicillin is a small nonimmunogenic molecule. Once degraded within the body, however, it forms a very reactive "penicilloyl" group, which can bind to serum proteins such as albumin to form penicilloyl-albumin complexes (Figure 7-7). The penicilloyl hapten can be recognized as a foreign epitope in some individuals and so provokes an immune response.

FIGURE 7-5 A molecular model of an antigen. This is an important influenza virus antigen called the hemagglutinin. It consists of two chains, one of which is lightly drawn so that the details of the other can be seen. The irregular surface forms characteristic shapes that can be recognized by the cells of the immune system. Influenza virus constantly changes the shape of this molecule, and this is denoted by the color coding. (Courtesy Dr. Fabian Glaser.)

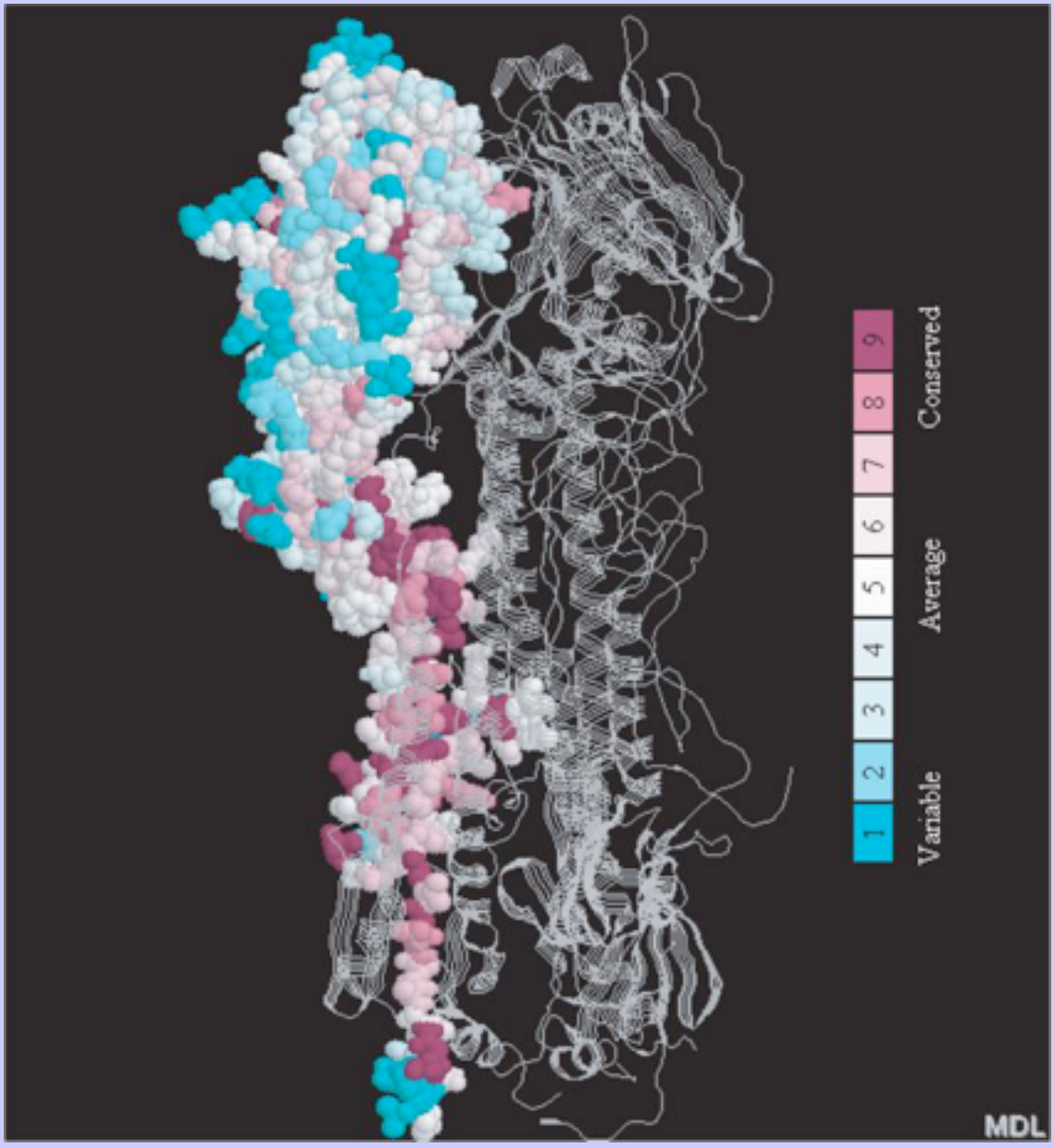


FIGURE 7-6 **A,** A typical hapten, in this case, dinitrophenol attached to a lysine side chain. **B,** When several haptens are attached to a peptide chain, they serve as new epitopes and will stimulate immune responses.

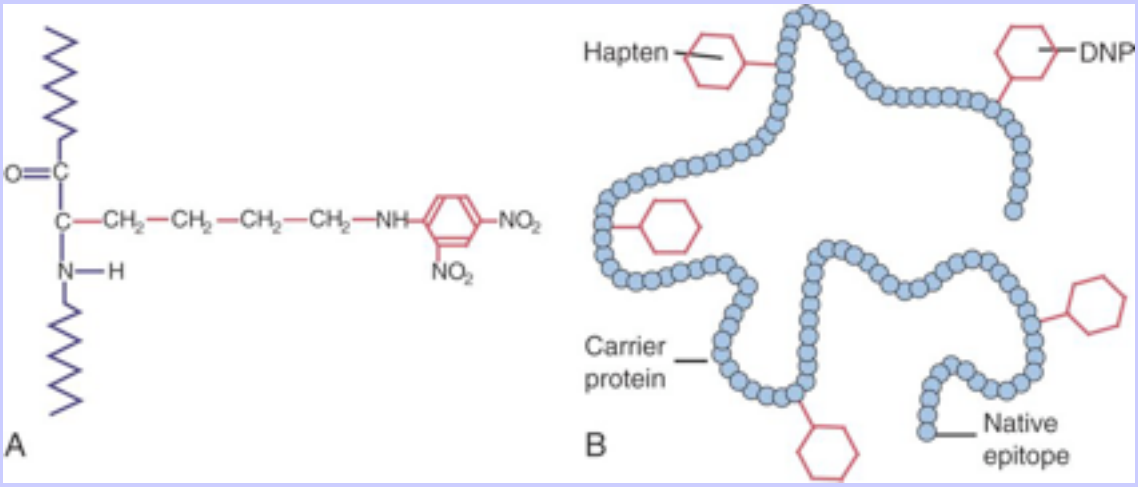


FIGURE 7-7 Penicillin as a hapten. Penicillin can break down in vivo by several different pathways. The most important derivative is a penicillenic acid that combines with amino groups in a protein such as serum albumin to form a penicilloyl-protein complex. This complex may provoke an immune response and result in a penicillin allergy.

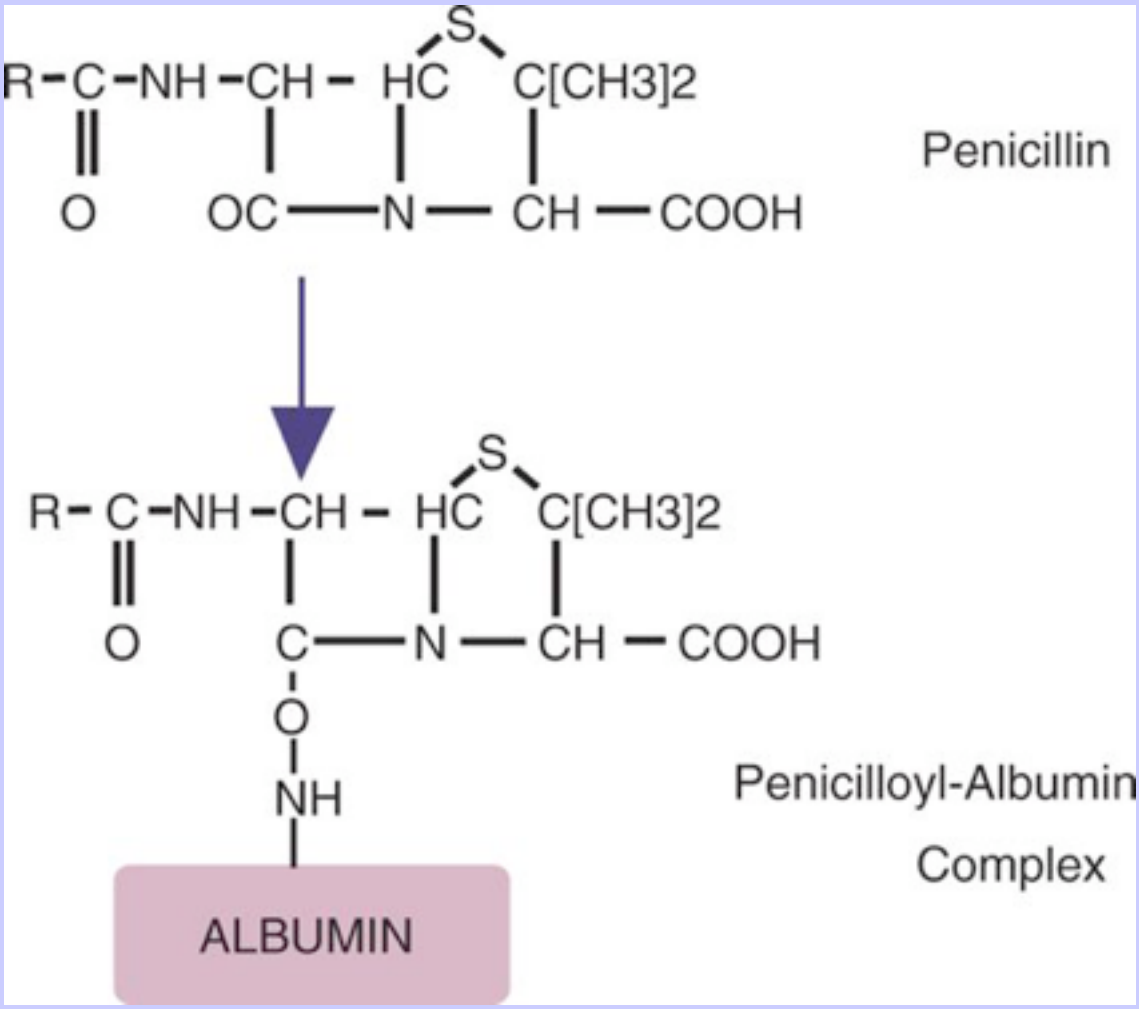
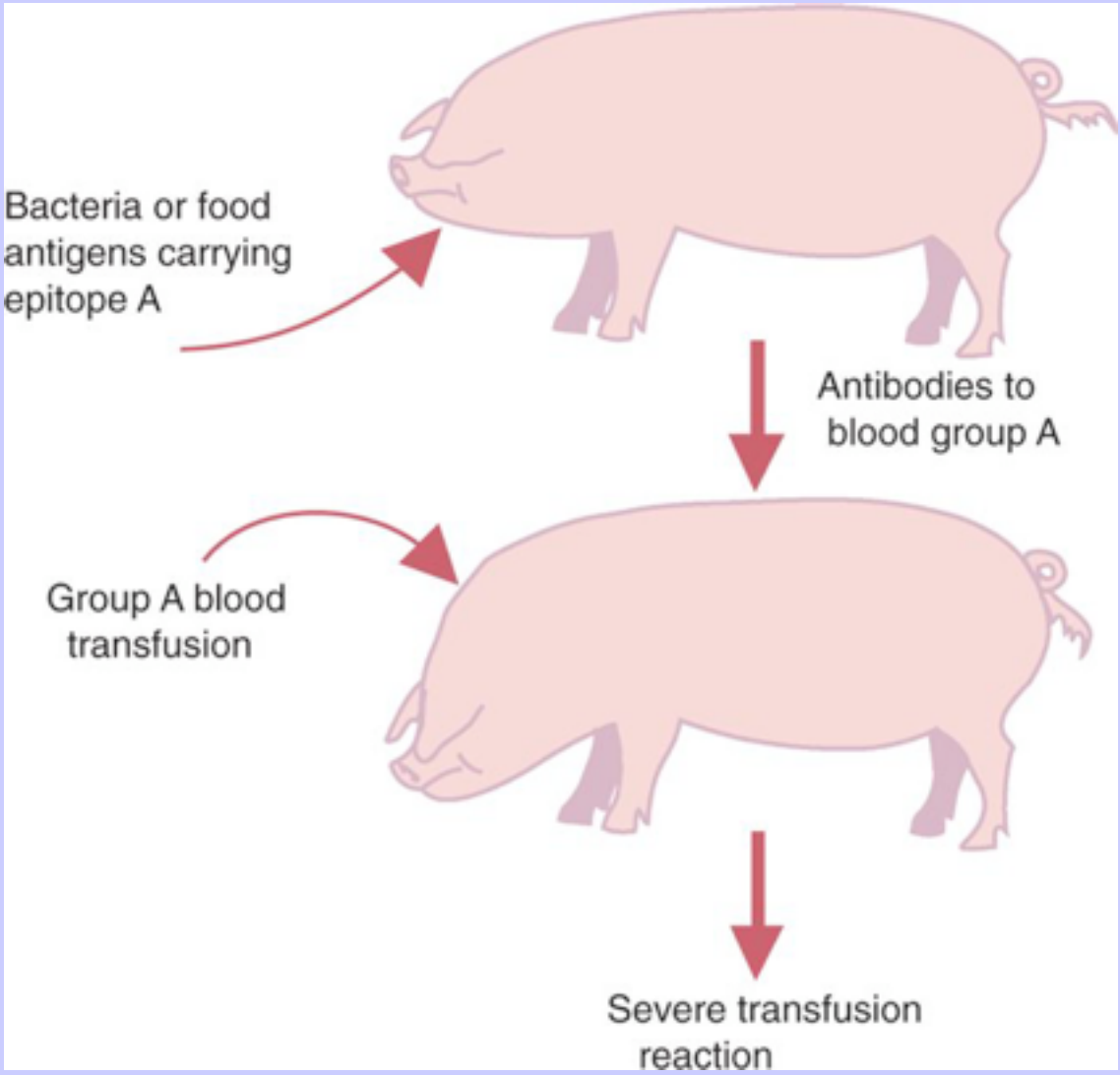


FIGURE 7-8 Food or bacterial antigens encountered in the diet carry epitopes that cross-react with blood group glycoprotein A. As a result, pigs of blood group O make antibodies to the A epitope despite never having received group A red cells. Should these animals be inadvertently transfused with A blood, they will suffer an immediate and severe transfusion reaction.



A second example of a naturally occurring reactive chemical that binds spontaneously to normal proteins and so acts as a hapten is the toxic component of the poison ivy plant *(Rhus radicans)*. The resin of this plant, called urushiol, will bind to any protein with which it comes into contact—including the skin proteins of a person who rubs against the plant. The modified skin proteins are then regarded as foreign and attacked by lymphocytes in a

manner similar to the rejection of a skin graft. The result is the uncomfortable skin rash called allergic contact dermatitis (see Chapter 28).

7.7 CROSS-REACTIONS

Identical or similar epitopes may sometimes be found on apparently unrelated molecules. As a result, antibodies directed against one antigen may react unexpectedly with an unrelated antigen. In another situation, the epitopes on a protein may differ in only minor respects from those on the same protein obtained from an animal of a related species. Consequently, antibodies directed against a protein in one species may also react in a detectable manner with the homologous or similar protein in another species. Both phenomena are called cross-reactions.

An example of a cross-reaction of the first type is seen when blood-typing. Many bacteria possess cell wall glycoproteins with carbohydrate side chains that are identical to those found on mammalian red blood cell glycoproteins. For example, some intestinal bacteria possess glycoproteins with A or B side chains on their cell walls (see Chapter 26). These glycoproteins are absorbed through the intestinal wall into the bloodstream and trigger an antibody response. For example, blood group glycoprotein side chain A is foreign to a pig of blood group O (Figure 7-8). Pigs of blood group O therefore develop antibodies that react with red cells from pigs of blood group A. These antibodies arise not as a response to previous immunization with group A red cells but following exposure to the glycoproteins that originate in the intestinal bacteria. Cross-reacting antibodies of this type are called heterophile antibodies.

Another example of cross-reactivity occurs between *Brucella abortus* and some strains of *Yersinia enterocolitica. Y. enterocolitica,* a relatively unimportant organism, may provoke cattle to make antibodies that cross-react with *B. abortus*. Since *Brucella*-infected animals are detected by testing for the presence of serum antibodies, a *Yersinia*-infected animal may be wrongly thought to carry *B. abortus* and so be killed. In another example, cross-reactivity occurs between the virus of feline infectious peritonitis (FIP) and the virus of pig-transmissible gastroenteritis (TGE). It is very difficult to grow the FIP virus in the laboratory. TGE virus, on the other hand, is readily propagated. By detecting antibodies to TGE in cats, it is possible to diagnose FIP without having to culture the FIP virus.

The second type of cross-reactivity, which occurs between related proteins, may be demonstrated in many different biological systems. One example is the method used to detect relationships between mammalian species. Thus antisera to bovine serum albumin cross-react well with sheep and goat serum albumin but react much more weakly with serum albumin from other mammals (Table 7-1). Presumably, this reflects the degree of structural similarity between the epitopes on serum proteins and is thus a useful tool in determining evolutionary relationships.

Table 7-1 The Degree of Cross-Reaction between a Specific Antibody (Antibovine Light Chain Antibodies) and Related Proteins (Light Chains) from Other Mammals

Cow	*Bos taurus*	100
Bison	*Bos bison*	100
Sheep	*Ovis aires*	100
Yak	*Pocphagus grunniens*	68
Goat	*Capra hircus*	68
Elk	*Cervus canadensis*	64
Buffalo	*Bubalus bubalus*	54
Reindeer	*Rangifer tarandus*	37
Human	*Homo sapiens*	17
Horse	*Equus caballus*	10
Rat	*Rattus rattus*	10
Mouse	*Mus musculus*	10
Pig	*Sus scrofa*	8
Camel	*Camelus dromedarius*	7

Data from Henning D, Nielsen K: *Vet Immunol Immunopathol* 34:235-243, 1992.

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